NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ISOBUTENE

(CAS NO. 115-11-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

December 1998

NTP TR 487

NIH Publication No. 99-3977

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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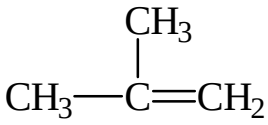
ABSTRACT



ISOBUTENE

CAS No. 115-11-7

Chemical Formula: C_4H_8 Molecular Weight: 56.10

Synonyms: γ-butylene, isobutylene, liquified petroleum gas, 2-methylpropene

Isobutene is produced during the fractionation of refinery gases or through the catalytic cracking of methyl-*t*-butyl ether. Isobutene is primarily used to produce diisobutylene, trimers, butyl rubber, and other polymers. In addition, it is used in the production of isooctane, high-octane aviation gasoline, methyl-*t*-butyl ether, and copolymer resins with butadiene and acrylonitrile. Isobutene was selected for evaluation because of the potential for human exposure due to its large production volume and the lack of adequate data on its carcinogenic potential. The toxicity and carcinogenicity of isobutene were determined in male and female F344/N rats and $B6C3F_1$ mice exposed to isobutene (greater than 98% pure) by inhalation for 14 weeks or 2 years. The mutagenicity of isobutene was assessed in *Salmonella typhimurium,* and the frequency of micronuclei was determined in the peripheral blood of mice exposed by inhalation for 14 weeks.

14-WEEK STUDIES

Groups of 10 male and 10 female F344/N rats and $B6C3F_1$ mice were exposed to isobutene at concentrations of 0, 500, 1,000, 2,000, 4,000, or 8,000 ppm 6 hours per day, 5 days per week, for 14 weeks. Concentrations greater than 8,000 ppm isobutene were not used because of the danger of explosion. All rats and mice survived to the end of the study. The final mean body weights and body weight gains of all exposed groups were similar to those of the chamber controls. No exposure-related gross lesions were observed in male or female rats or mice at necropsy. Microscopically, minimal hypertrophy of goblet cells lining the nasopharyngeal duct in the most caudal nose section was observed in some rats in each exposed group of males and females.

2-YEAR STUDIES

Based on the lack of significant exposure-related toxicologic effects in the 14-week rat and mouse studies, 8,000 ppm was selected as the highest exposure concentration in the 2-year studies. Concentrations of 0, 500, 2,000, and 8,000 ppm were selected for rats and mice with the 500 and 2,000 ppm selection based on published metabolic elimination rates for Sprague-Dawley rats and $B6C3F_1$ mice.

Rats

Groups of 50 male and 50 female F344/N rats were exposed to isobutene at concentrations of 0, 500, 2,000, or 8,000 ppm 6 hours per day, 5 days per week, for 105 weeks. Survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed groups were generally similar to those of the chamber controls throughout the study.

2-Hydroxyisobutyric Acid — Biomarker of Exposure

2-Hydroxyisobutyric acid (HIBA), the major urinary metabolite of isobutene, was measured in the urine of male and female rats as an indicator of isobutene exposure at 6, 12, and 18 months. The amount of HIBA excreted increased with increasing exposure concentration. However, when HIBA concentration was normalized to isobutene exposure concentration, the relative amount of HIBA excreted decreased with increasing exposure concentration, implying nonlinear kinetics.

Pathology Findings

The incidence of thyroid gland follicular cell carcinoma in male rats exposed to 8,000 ppm was increased compared to the chamber control group and exceeded the historical control range. The incidences of hyaline degeneration of the olfactory epithelium were marginally increased in exposed rats; however, the severities of hyaline degeneration increased with increasing exposure concentration in males and females.

Mice

Groups of 50 male and 50 female B6C3F_1 mice were exposed to isobutene at concentrations of 0, 500, 2,000, or 8,000 ppm 6 hours per day, 5 days per week, for 105 weeks. Survival of exposed males and females was similar to that of the chamber controls. Mean body weights of exposed mice were generally similar to those of the chamber controls throughout the study except for female mice exposed to 2,000 or 8,000 ppm, which weighed slightly less than chamber controls from about week 52 until week 92.

2-Hydroxyisobutyric Acid — Biomarker of Exposure

HIBA was measured in the urine of male and female mice as an indicator of isobutene exposure at 6, 12, and 18 months. The amount of HIBA excreted increased with increasing exposure concentration. However, when HIBA concentration was normalized to isobutene exposure concentration, the relative amount of HIBA excreted decreased with increasing exposure concentration, implying nonlinear kinetics.

Pathology Findings

The incidences of hyaline degeneration of the respiratory epithelium in all groups of exposed males and females were significantly greater than those in the chamber control groups. The incidences of hyaline degeneration of the olfactory epithelium in 2,000 and 8,000 ppm mice were greater than those in the chamber controls.

GENETIC TOXICOLOGY

Isobutene was not mutagenic in any of four strains of *S. typhimurium*, with or without S9 metabolic activation, and no increase in the frequency of micronucleated erythrocytes was seen in peripheral blood of male or female mice treated with isobutene by inhalation for 14 weeks.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of isobutene in male F344/N rats based on an increased incidence of follicular cell carcinoma of the thyroid gland. There was *no evidence of carcinogenic activity* of isobutene in female F344/N rats or male or female B6C3F_1 mice exposed to 500, 2,000, or 8,000 ppm.

Exposure to isobutene by inhalation for 2 years resulted in increased incidences and/or severities of nasal lesions including hyaline degeneration of the olfactory epithelium in male and female rats and mice and hyaline degeneration of the respiratory epithelium in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Isobutene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in air	0, 500, 2,000, or 8,000 ppm	0, 500, 2,000, or 8,000 ppm	0, 500, 2,000, or 8,000 ppm	0, 500, 2,000, or 8,000 ppm
Body weights	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group	Exposed groups similar to chamber control group	2,000 and 8,000 ppm groups slightly less than chamber control group
Survival rates	7/50, 5/50, 6/50, 8/50	23/50, 19/50, 33/50, 22/50	28/50, 32/50, 27/50, 28/50	32/50, 31/50, 39/50, 33/50
Nonneoplastic effects	Nose: severity of olfactory epithelial hyaline degeneration (1.3, 1.4, 2.2, 2.6)	Nose: severity of olfactory epithelial hyaline degeneration (1.5, 2.4, 2.8, 2.8)	Nose: respiratory epithelial hyaline degeneration (6/50, 19/49, 29/50, 39/48); olfactory epithelial hyaline degeneration (6/50, 7/49, 16/50, 17/48)	Nose: respiratory epithelial hyaline degeneration (21/47, 39/50, 41/49, 48/50); olfactory epithelial hyaline degeneration (17/47, 19/50, 24/49, 27/50)
Neoplastic effects	Thyroid gland: follicular cell carcinoma (1/48, 0/48, 0/48, 5/50)	None	None	None
Level of evidence of carcinogenic activity	Some evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535, with and without S9			
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:	Negative in male and female mice			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on isobutene on 10 December 1997 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 10 December 1997, the draft Technical Report on the toxicology and carcinogenesis studies of isobutene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of isobutene by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *some evidence of carcinogenic activity* in male F344/N rats and *no evidence of carcinogenic activity* in female F344/N rats or male or female B6C3F_1 mice.

Dr. Bailer, a principal reviewer, agreed in principle with the proposed conclusions. He said that because there were neoplasms at only one site in the 8,000 ppm group in just one species, he would be comfortable as well with *equivocal evidence of carcinogenic activity* in male rats. Dr. Bailer said that he would like to see information on typical levels of human exposure for the potentially exposed workers. Dr. Roycroft responded that there were no human exposure data, and only a limited amount of data in the literature provides even a hint of how people might be exposed to isobutene; for example, 1% of gasoline may be isobutene. Dr. G.W. Lucier, NIEHS, reported that the NIEHS/NTP has recently established an interagency agreement with the Centers for Disease Control and Prevention, to provide information on exposure assessment for chemicals of interest to the NTP, including those evaluated by this Subcommittee.

Dr. Medinsky, the second principal reviewer, agreed with the proposed conclusions. She complimented the NTP for the use of pharmacokinetic data. Dr. Medinsky said it would be useful to include a metabolic scheme for isobutene so that the reader could readily see where the biomarker, 3-hydroxyisobutyric acid, fits in the metabolic fate of isobutene relative to what in fact might be the toxic metabolite for this chemical. Dr. Roycroft said that a metabolic scheme could be included, that the monoepoxide is a putative metabolite, and that a metabolic modeling effort has been started on isobutene to predict some blood concentrations of the epoxide.

Dr. Belinsky, the third principal reviewer, agreed with the proposed conclusions. He thought it interesting that there was no apparent precursor lesion, hyperplasia, for the thyroid gland neoplasms. Dr. R.A. Herbert, NIEHS, commented that hyperplasia can be considered a preneoplastic lesion for thyroid gland neoplasms and that this has been seen in other studies; however, in this study, the incidences of follicular cell hyperplasia were not significantly increased.

Dr. Bailer moved that the Technical Report on isobutene be accepted with the revisions discussed and the conclusions as written for male rats, *some evidence of carcinogenic activity*, and for female rats and male and female mice, *no evidence of carcinogenic activity*. Dr. Medinsky seconded the motion, which was accepted unanimously with eight votes.